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Histological Responses of Port Wine Stains in Brown Skin After 578 nm Copper Vapor Laser Treatment

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Background and Objective: **The object of this study is to characterize the effects of epidermal melanin in brown skin on selective vessel damage by copper vapor laser radiation in port wine stain (PWS).**

Study Design/Materials, and Methods: **We observed the histological changes of PWS in Korean patients who received copper vapor laser (578 nm) treatment over a range of energy densities (6–14 J/cm^2) and exposure durations (30–200 ms). The nitroblue tetrazolium chloride (NBTC) staining method was used to differentiate between the blue-stained viable cells and the unstained thermally damaged cells.**

Results: **With Fontana-Masson stain, we found that Korean skin has more epidermal melanin than Caucasian skin. For energy densities greater than 6 J/cm^2, epidermal damage was observed. At 6 and 8 J/cm^2, the damage to the dermis was localized to the blood vessels and the perivascular tissue. The connective tissue between damaged vessels and epidermis was still viable. Energy densities above 10 J/cm^2 produced a diffuse thermal necrosis. We conclude that vascular selectivity without epidermal damages cannot be achieved with a 50 ms exposure at 578 nm in the brown skin of Koreans. The energy density for clinical minimal whitening was 6–8 J/cm^2, and the maximum penetration depth of these energy densities was 0.4 mm. We also found that the epidermal damage increased with increasing pulse widths at a fixed energy density (10 or 8 J/cm^2) while the severity and depth of vascular damage decreased. These findings suggest that it is best to treat PWS with a copper vapor laser at the minimal pulse width and maximal power output possible at given energy density.**

Conclusion: **We have demonstrated that the copper vapor laser treatment of PWS in the brown skin is not as selective as in white skin because of epidermal melanin.**

Key words: energy density, epidermal damage, exposure duration, Korean, melanin, pulse width, copper vapor laser

INTRODUCTION

The selective tissue damage by laser radiation is influenced by wavelength, exposure duration (pulsewidth), and exposure dose (energy density) [1]. Optimal laser coagulation of port wine stain (PWS) depends on the wavelength selective absorption of radiation by oxyhemoglobin (HbO_2), the target chromophore. Yellow laser light around 577 nm matches one of the absorption peaks of oxyhemoglobin and thus is selectively absorbed by blood vessels, provided that the energy is delivered briefly enough to confine thermal damage to the vessel wall [2].

Recently, encouraging results in the treatment of PWS have been obtained using yellow

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laser light at around 577 nm, that is, the light emitted from pulsed dye laser (PDL) or copper vapor laser (CVL) [3–6]. But most of these results were obtained from Caucasian patients who had white skin.

For effective treatment, absorption by competing epidermal melanin, which results in epidermal damage, should be minimal. Although epidermal melanin absorbs less yellow light than argon laser radiation, it still acts as a competing chromophore at wavelengths around 577 nm [7,8]. The overlying epidermal pigment layer, therefore, represents a potential barrier through which 577 or 578 nm laser radiation must pass to reach the underlying microvasculature. Thus, it is possible that it is more difficult to produce selective vascular damage in the PWS with pigmented skin, such as in Koreans, using 578 nm pulses.

In this study we observed the immediate histologic changes caused by CVL radiation in PWS of Korean patients over a range of several energy densities and exposure durations. To characterize the effects of epidermal melanin pigmentation on selective vascular injury, we compared our results with those obtained by other authors using Caucasian patients [6,9]. The nitroblue tetrazolium chloride (NBTC) staining method was used to differentiate between viable (blue-stained) cells and thermally damaged (unstained) cells [9–11].

MATERIALS AND METHODS

Patients

Nine informed, consenting, Korean patients with PWS (4 females, 5 males) were treated with a copper vapor laser (CVL). Their ages ranged from 17 to 45 years (mean 27 years). The constitutive skin color of Koreans can be categorized into skin phototype III, IV, or V [12]. All PWS were located on the face, including the anterior portion of the scalp. Six lesions were purple in color, and the remainder were reddish-purple or red. None of the patients had received previous treatment.

Copper Vapor Laser Treatment

A CVL (VCM—10, Visiray Co., Australia) was used. It produces two wavelengths, which can be emitted either separately or together: green (511 nm) and yellow (578 nm). The yellow wavelength is adjustable up to a maximum of 2.5 W. This CVL produces a train of pulses with a pulsewidth of 24 ns. Approximately 12,000 pulses/s (12 kHz) are emitted. In this study, we used the yellow wavelength (578 nm) in two types of experimental conditions. In the first, we varied the energy density from 6 to 14 J/cm^2 and kept the exposure duration constant at 50 ms. In the second, we varied the exposure duration from 30 to 200 ms and kept a fixed energy density at either 8, 10, or 12 J/cm^2. After shaving off all hair, the skin around the hairline was exposed to several exposure doses of laser light. All treatments were performed without anesthesia.

Laser light was delivered by the Hexascan (Prein & Partners, France), an automated delivery system using a 1 mm diameter spot [13]. This system consists of a microprocessor-controlled scanning handpiece that is connected via a control module to the fiber-optic delivery system of the laser. An automated program places pulses of laser energy, separated by 50 ms, in a precise, nonadjacent scanning pattern to allow dissipation of undesired thermal energy between exposures. The desired energy fluences were selected on the control panel of the Hexascan.

Histochemical Procedures

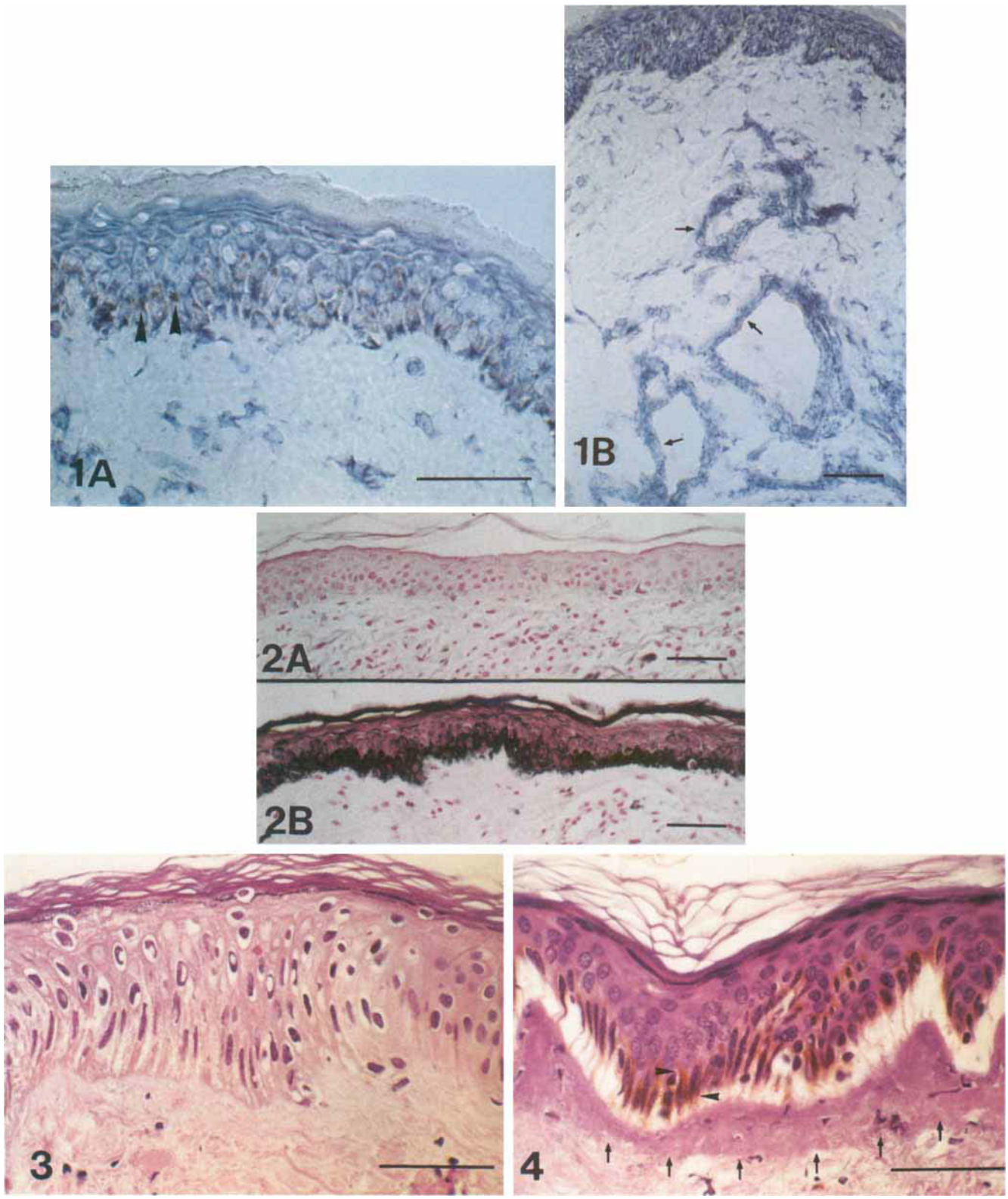
Three or four 3-mm punch biopsy specimens were obtained from each patient at predetermined exposure doses under local anesthesia with 2% lidocaine. All biopsy specimens were obtained within 15 min after laser treatment and split for routine H & E, Fontana-Masson staining, and NBTC staining. The tissues for NBTC staining were snap-frozen in liquid nitrogen and stored at

Fig. 1. NBTC staining of normal skin. **A:** All viable epidermal and dermal cells present a blue stain with fine cytoplasmic pigment granules. The horny layer and the dermal collagen remain unstained. Melanin pigments are easily observed in the basal cell layer (arrowheads). **B:** Blood vessels in the dermis are well delineated (arrows). Bars in A and B are 50 μm.

Fig. 2. Fontana-Masson staining of Caucasian (**A**) and Korean skin (**B**). Bars in A and B are 50 μm.

Fig. 3. Copper vapor laser, 6 J/cm^2. Morphological changes of epidermis with spindling of basal cells, vertical elongation of nuclei, and vacuoles in the cytoplasms. H & E stain. Bar, 50 μm.

Fig. 4. Copper vapor laser, 8 J/cm^2. Diffuse morphological changes in epidermis with elongation and vesiculation of nuclei and separation of dermo-epidermal junction. Basophilic degeneration of the papillary dermis (arrows) is observed. Epidermal melanin pigments (arrowheads) are abundant in the basal cell layer. H & E stain. Bar, 50 μm.



Figs. 1-4.

TABLE 1. Degree of Epidermal and Dermal Damage in Port Wine Stains in Brown Skin 15 Minutes After Copper Vapor Laser Treatment (578 nm) According to Increasing Energy Densities With Fixed Exposure Duration

Patients	Exposure duration (ms)	Power output (W)	Energy density (J/cm^2)	Epidermal damage		Dermal damage[b]	
				Morphology[a]	Viability[b]	Vascular	Nonvascular
1	50	0.9	6	+ /−	−	−	−
	50	1.3	8	+ + /+	+	+ +	−
	50	1.6	10	+ + /+	+ + +	+ + +	+ + +
	50	1.9	12	+ + /+	+ + +	+ + +	+ + +
2	50	0.9	6	+ /+	−	+ + +	−
	50	1.3	8	+ /+	+	+ + +	−
	50	1.9	12	+ + /+	+ +	+ + +	+ + +
3	50	0.9	6	+ /−	−	−	−
	50	1.3	8	+ + /+	+	+ + +	−
	50	1.9	12	+ + + /+	+ + +	+ + +	+ + +
4	50	1.3	8	+ + /+	+	+ + +	−
	50	1.6	10	+ + /+	+	+ + +	+ + +
	50	1.9	12	+ + /+	+	+ + +	+ + +
	50	2.2	14	+ + /+	+	+ + +	+ + +

[a]Morphological changes in H & E stained sections/presence of subepidermal cleft: −, no morphological change; +, only morphological changes in basal cell layer; + +, mild to moderate, diffuse morphological changes; + + +, severe, diffuse morphological changes.
[b]Cell viability in NBTC stained sections: −, only viable cell; +, some cells with weaker NBTC stain; + +, marked number of weaker NBTC stain and devitalized cells; + + +, diffuse coagulation necrosis with devitalized unstained cells.

−70°C. All tissues were sectioned at multiple levels by means of a cryocut microtome (Reiter-Jung Cryocut 1800, Reiter-Jung, Germany). The specimens for NBTC staining were processed as previously described [10].

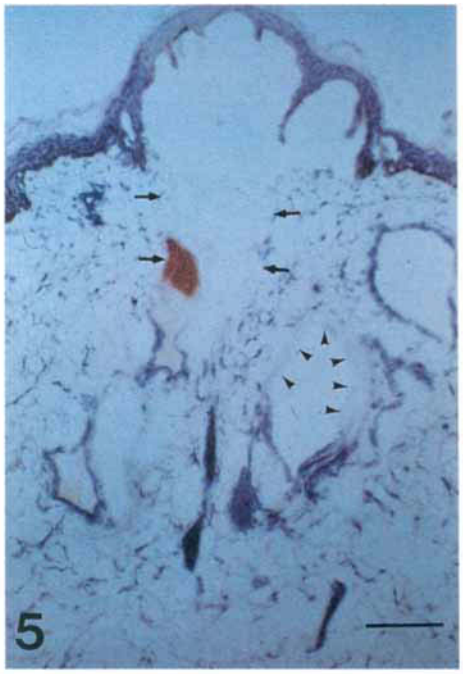
RESULTS

With NBTC staining, all viable cells in the epidermis and the dermis presented a blue stain with fine cytoplasmic pigment granules, sparing the nuclei. The horny layer and the dermal collagen remained unstained. The normal structures of the epidermis and blood vessels were well delineated (Fig. 1). Melanin pigment in the basal cell layer could be observed clearly in the NBTC-stained sections. Fontana-Masson stained sections showed that Korean skin has more melanin pigment in the epidermis than Caucasian skin (Fig. 2). There was less melanin pigment in the epidermis of patient 4 than in that of the other three patients in the Fontana-Masson stained sections (data not shown).

We have observed the histological changes resulting from increasing energy densities of 6, 8, 10, 12, and 14 J/cm^2 with a fixed exposure duration of 50 ms (Table 1). For energy densities of 6 J/cm^2 and above in H & E stain, epidermal cells showed morphological changes of the basal cell layer (spindling of cells, vertical elongation of nuclei, and vacuoles in the cytoplasms) (Fig. 3). At 8 J/cm^2, diffuse morphological changes in the whole epidermis were observed with elongation and vesiculation of nuclei, and basophilic degeneration of the upper dermis (Fig. 4). Separation of the dermoepidermal junction was also observed at energy densities of 6 and 8 J/cm^2 (Fig. 4). The severity of these morphological changes and the length of damaged epidermis increased at 10 and 12 J/cm^2.

With NBTC staining, all epidermal cells appeared viable at 6 J/cm^2. At 8 J/cm^2, some epidermal cells showed weaker NBTC stain (Fig. 5). Above 10 J/cm^2, there was a marked weakening of NBTC stain and diffuse coagulation necrosis of epidermis (Fig. 6), except in patient 4. In this patient, some epidermal cells showed weaker NBTC satin at energies up to 14 J/cm^2.

In the underlying dermis at 8 J/cm^2, thermal injury was limited to blood vessels in the papillary and midreticular dermis. In addition, a cuff of perivascular collagen was damaged. The connective tissue between the overlying epidermis and perivascular necrotic tissue remained viable (Fig. 5). Some superficial dermal vessels and the epidermis were so close that the connective tissue between the epidermis and vessels showed diffuse necrosis (Fig. 5). The energy density of 6 J/cm^2 also caused thermal injury confined to the superficial dermal vessels in patient 2, but not in patients 1 and 3. From 10 J/cm^2 diffuse coagulation necrosis of connective tissue between target vessels and the epidermis was observed (Fig. 6).



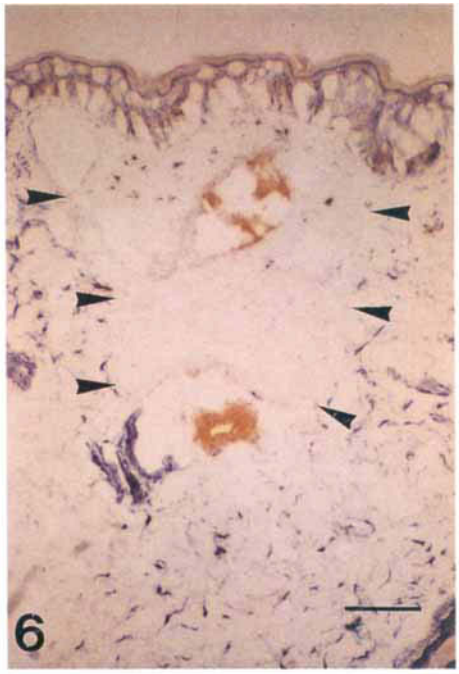


Fig. 5. Copper vapor laser, 8 J/cm^2. Some epidermal cells shows weaker NBTC stain. In the underlying dermis, thermal injury was confined to blood vessels in the mid-dermis (arrowheads). A superficial dermal vessel and the epidermis were so close that the connective tissue between the overlying epidermis and the damaged vessel showed diffuse necrosis (arrows). NBTC stain. Bar, 100 μm.

Fig. 6. Copper vapor laser, 10 J/cm^2. The stainability of the epidermal cells is decreased markedly and diffuse coagulation necrosis of connective tissue between the target vessels and the epidermis is observed (arrowheads). NBTC stain. Bar, 100 μm.

Fig. 7. NBTC staining of the pretreated **(A)** and 2 months post-treated (14 J/cm^2) PWS **(B)**. The epidermis of post-treated skin showed a normal appearance. The number and size of the vascular structures are reduced and replaced by collagen. NBTC stain. Bar, 100 μm.

The energy density for minimal clinical whitening ranged 6–8 J/cm^2 in all four patients. At these energy densities, morphological changes of epidermal cells have already been produced. The depth of vascular injury was measured using light microscopy from the dermo-epidermal junction to the innermost vertical border between unstained and blue-stained tissue. At the energy density of 8 J/cm^2 the range of thermal injury depth was 0.25–0.4 mm. Increasing injury depths correlated with increasing energy densities (Table 2).

We have also observed the histological changes caused by increasing pulse widths at

TABLE 2. Depth of Dermal Damage in Port Wine Stains in Brown Skin After Copper Vapor Laser (578 nm) Treatment

Exposure duration (ms)	Energy density (J/cm^2)	Depth of damage[a] (mm)
50	6	Epidermal to 0.13
50	8	0.25–0.4
50	10	0.35–0.5
50	12	0.28–0.55

[a]Measured from the dermo-epidermal junction in the NBTC stained sections.

fixed energy densities of 12, 10, and 8 J/cm^2, (Table 3). At the energy density of 12 J/cm^2 there were no significant differences in the severity of epidermal and dermal damages between exposure durations of 50, 100, 150, and 200 ms. But at 10 and 8 J/cm^2, increased damage to the epidermis and decreased damage to dermal blood vessels was observed with increased in pulsewidth (30–140 ms in patient 6 and 50–150 ms in patient 7). The depth of vascular injury also decreased with increasing pulsewidth.

Lastly, we observed the histological changes of PWS 1–2 months after CVL treatment in two patients who were treated with an energy density of 14 J/cm^2 and a pulsewidth of 56 ms (Table 4). The epidermis looked normal and the number and size of the vascular structures in the upper and mid-dermis were reduced markedly and replaced by collagen (Fig. 7).

DISCUSSION

Histochemical NBTC staining for determination of cell viability in thermally damaged skin has been established previously [10,11]. Reduction of NBTC by nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase) produces an intense blue cytoplasmic pigment in frozen tissue sections. The activity of this enzyme has been shown to subside immediately upon cell death. Because of the marked color difference between viable and devitalized structures, the NBTC method provides a precise and rapid picture of the extent of thermally induced damage. It allows identification of laser injury by direct demonstration of vessel wall necrosis and the extent of perivascular connective tissue damage [9].

In our study morphological changes in basal cells appeared starting at 6 J/cm^2 and loss of viability of the epidermal cells appeared starting at 8 J/cm^2. But in the dermis, at energy densities of 6 and 8 J/cm^2 damage was confined to the blood vessels and surrounding collagen. The stromal tissue between damaged vessels and the epidermis was still viable. Energy densities of 10 J/cm^2 and higher produced a wedge-shaped diffuse coagulation necrosis. Therefore, it is apparent that vascular selectivity without epidermal damage cannot be achieved at 578 nm in the brown skin of Koreans.

Neumann et al. have studied the histochemical changes occurring 15 minutes after CVL treatment of Caucasian patients with port wine stains using the NBTC method [9]. They used a range of energy densities of 8–32 J/cm^2 at 578 nm with a maximum power output of 1.3 W. In their study, exposure of PWS skin to 8–12 J/cm^2 (its corresponding pulsewidths were in the range of 50–74 ms) did not alter the integrity of epidermal cells but could cause damage confined to blood vessels and surrounding collagen, whereas higher fluences ($\geq$15 J/cm^2) produced nonspecific tissue damage.

Korean skin looks brownish and is more heavily pigmented than Caucasian skin. The skin phototype of Koreans is usually classified into types III, IV, and V. Our findings demonstrate that epidermal damage in brown skin caused by CVL radiation occurs at lower energy densities than in Caucasian skin, and the range of energy densities that shows vessel selectivity in the dermis is much narrower than that for Caucasian skin [6,9].

These different histological responses to CVL for Caucasian and Korean skins arise from the differing degrees of melanization of the skin. The cutaneous target of CVL radiation is the oxyhemoglobin of intravasal erythrocytes, and results in selective destruction of blood vessels with preservation of the overlying epidermis. However, epidermal melanin acts as a competing chromophore, and the target specificity of yellow light laser is influenced by variations in epidermal pigmentation [7]. Caucasian skin has less total melanin than Mongoloid skin [14]. We also found that in the Fontana-Masson stain, melanin pigment was heavily present in the low spinous layer of epidermis of Korean skin compared with Caucasian skin. The melanin pigment absorbs the laser light and generates heat, which is conducted to the adjacent epidermis and underlying dermis, resulting in nonspecific coagulation necrosis of the epidermis and upper dermis. In our study, we observed that diffuse necrosis of the epidermis started at 10 or 12 J/cm^2 in patients 1, 2, and 3.

TABLE 3. Degree of Epidermal and Dermal Damage in Port Wine Stains in Brown Skin 15 Minutes After Copper Vapor Laser Treatment (578 nm) According to Increasing Exposure Duration

Patients	Exposure duration (ms)	Power output (W)	Energy density (J/cm^2)	Epidermal damage		Dermal damage[b]		Depth of Damage (mm)
				Morphology[a]	Viability[b]	Vascular	Nonvascular	
5	50	1.9	12	+ + +/+	+ +	+ + +	+ + +	0.28
	100	0.9	12	+ + +/+	+	+ + +	+ + +	0.28
	150	0.6	12	+ + +/+	+ +	+ + +	+ + +	0.25
	200	0.5	12	+ + +/+	+ + +	+ + +	+ + +	0.3
6	30	2.5	10	+ +/+	+	+ + +	+ + +	0.58
	70	1.1	10	+ +/+	+ +	+ + +	+ + +	0.3
	140	0.6	10	+ + +/+	+ +	+	+ + +	0.18
7	50	1.3	8	+/+	−	+ + +	−	0.32
	100	0.6	8	+ +/+	+	+	−	0.13
	150	0.4	8	+ +/+	+	+	−	0.10

[a,b]Same as in Table 1.

TABLE 4. Degree of Epidermal and Dermal Damage in Port Wine Stains in Brown Skin 1 or 2 Months After Copper Vapor Laser Treatment (578 nm)

Patients	Exposure duration (ms)	Power output (W)	Energy density (J/cm^2)	Epidermal damage		Dermal damage[b]	
				Morphology[a]	Viability[b]	Vascular	Nonvascular
8	56	2.0	14	− /−	−	−	−
9	56	2.0	14	− /−	−	−	−

[a,b]Same as in Table 1.

But in patient 4, due to less melanin pigmentation, diffuse necrosis of the epidermis did not occur until 14 J/cm^2. These results suggest that lasers that emit yellow light, such as CVL and PDL, cannot produce the same treatment results with PWS in pigmented skin as in white skin.

In our study at 8 J/cm^2 with CVL, the connective tissue stroma between vessels and overlying epidermis was viable. This indicates that CVLs are different from argon lasers, since argon lasers produce diffuse necrosis extending from the epidermis [11]. Although the CVL epidermal damage was induced in all patients in this study, unlike with the argon laser [2,15], histological findings at 1–2 months in patients 8 and 9 showed that this damage was not permanent.

In addition to wavelength, the laser exposure duration also affects the extent to which heat will remain confined to the target vessel during exposure. It is known that if the exposure duration is long compared with the time required for diffusion of heat to surrounding structures, thermal damage will be extensive and nonspecific, regardless of how specifically the laser light is absorbed and heats a target structure [1]. Neumann et al. have reported that with a 50–74 ms exposure duration with CVL, selective vessel damage was observed in Caucasian skin. But in their study the increase in pulsewidth caused an increase in energy density. In our study, we compensated for this increase by reducing power output so that energy density was held constant. Our findings demonstrate that with increasing exposure duration, the damage to epidermal cells increases gradually. On the other hand, the severity of vascular damage and the depth of collagen damage decreased with increasing exposure duration. In our study these decreased thermal effects in the dermis might have resulted from the decreased power outputs with increasing exposure duration. With longer pulsewidths and lower power outputs, the heat diffuses away and therefore lower temperatures are reached, which may explain the lower depth of damage. This suggests that to achieve the best treatment results with CVL, it is necessary to treat with the maximal power output and minimal pulsewidth possible at a given energy density.

In recent CVL studies, the threshold fluences for clinical whitening in Caucasian skin were found to lie at 17–25 J/cm^2 [6] or 15–20 J/cm^2 [9]. Compared with whitening threshold fluences of 6–8 J/cm^2 in our study, those for white skin are very high. It is already known that immediate clinical whitening is always indicative of epidermal coagulation necrosis due to absorption by epidermal melanin [4,9]. Epidermal melanin, abundant in brown skin, may play an important

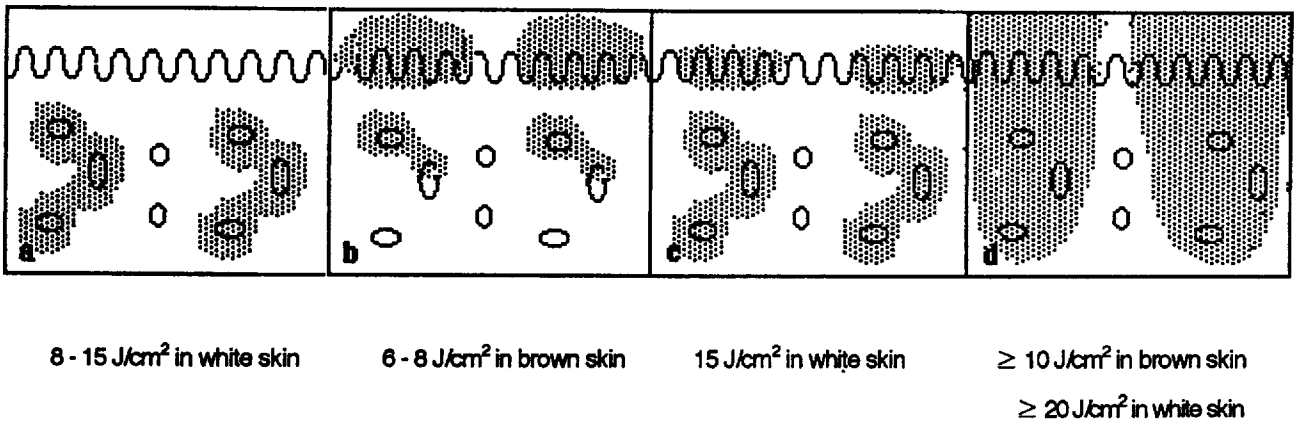


Fig. 8. Comparison of epidermal and dermal damage (shading) in port wine stains after copper vapor laser (578 nm) treatment in the white and brown skin. Selective damage to dermal vessels and perivascular tissue **(a)**, viable connective tissue between epidermis and vessels **(b,c)**, and nonspecific diffuse necrosis **(d)**. The data in white skin are cited from Neumann and Knobler [9].

role in reducing the whitening threshold fluences in our study.

In our study the penetration depth was found to depend on energy density and power output in a fixed pulsewidth (50 ms). With an energy density of 8 J/cm^2, a penetration depth of up to 0.4 mm into the dermis could be achieved. In spite of the melanin pigment in brown skin, these results did not show a significant difference from the depth in Caucasian skin [9]. The increased power output of CVL used in our study resulted in an increase of penetration of sufficient laser light to cause necrosis.

In summary we have demonstrated that CVL treatment of PWS in the brown skin is not as selective as in white skin because of epidermal melanin (Fig. 8).

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